

Detection of Estrogen Receptor Alpha in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Serum: Normal Horse Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 008-000-001

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Mouse Anti-ER (ER1D5) Antibody

Beckman Coulter, Inc.

Fullerton, CA 92834

www.beckmancoulter.com

1-800-458-5060

Catalog # IM1545

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdpharma.com

1-877-232-8995

Catalog # 557273

Secondary Antibody: Biotinylated Horse Anti-Mouse IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-2001

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Female reproductive tract
Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* _____

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

6. Block with 10% Normal Horse Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:50 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp Date _____

For negative control slides, dilute the protein concentration of the mouse IgG1 serum to match that of the primary antibody, if necessary. Make a 1:50 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the horse anti-mouse secondary antibody at a 1:1000 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Vectastain R.T.U Elite Label and incubate for 30 minutes at room temperature.

Exp. Date _____ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

14. Apply the DAB chromagen. Incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # _____ Exp. Date _____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip